

## The Brief Case

(For answers to the self-assessment questions and take-home points, see page 2406 in this issue [doi:10.1128/JCM.00213-16].)

### Cryptosporidiosis in a Severely Immunocompromised HIV Patient

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#### CASE

A 52-year-old male with AIDS was admitted to the University of Utah Hospital with chronic (>3-month duration) watery diarrhea. The patient had been diagnosed with HIV infection 3 years prior to admission but had been noncompliant with antiviral therapy since primary diagnosis. Three months prior to admission (at the time of the diarrhea onset), his CD4<sup>+</sup> cell count was critically low (6 cells/ $\mu$ l), he had an elevated viral load (~54,000 copies/ml), and he was displaying rapid deterioration of overall health. The patient also suffered from multiple other known viral complications attributable to his severe immunosuppression, including chronic cytomegalovirus (CMV) retinitis and recurrent anogenital lesions caused by herpes simplex virus 2 (HSV-2).

The patient was severely malnourished and hypokalemic at admission (potassium level, <1.6 mmol/liter). The stools (>20 per day) were nonbloody and devoid of mucus, and inflammation was indicated by the presence of lactoferrin in the stool. Routine stool cultures were negative for *Shigella*, *Salmonella*, *Campylobacter*, *Yersinia*, *Aeromonas*, *Plesiomonas*, and *Vibrio*. Shiga-like-toxin-producing *Escherichia coli* was not detected by antigen detection, and *Clostridium difficile* was not detected by real-time PCR. CMV colitis was excluded on the basis of negative results for immunohistochemical staining of colonoscopy-obtained biopsy samples. A single microscopic examination of stool for ova and protozoal parasites (O&P detection), consisting of a concentrated wet-mount preparation, a trichrome stain, a modified trichrome stain, and a modified acid-fast (MAF) stain, gave negative results for gastrointestinal parasites and microsporidia. The result for fecal antigen detection for *Cryptosporidium* was also negative. Additional testing with a multiplex real-time PCR panel for *Entamoeba histolytica*, *Giardia*, *Cryptosporidium parvum*/*Cryptosporidium hominis*, *Dientamoeba fragilis*, and *Cyclospora cayetanensis* gave a positive result for the presence of *Cryptosporidium* DNA. This result was discrepant with those for both antigen detection and microscopy but was repeatedly confirmed and eventually verified by sequencing of the amplicon.

To investigate this discrepancy and attempt to provide a clinical clarification of the test results, the MAF-stained slide was reexamined for the presence of *Cryptosporidium* oocysts. No oocysts were seen upon review. Two additional slide preparations from the original specimen were stained and examined and revealed rare oocysts with characteristic fuchsia staining, size (5- $\mu$ m diameter), and morphology consistent with this protozoan (Fig. 1). Several "ghost cells" were also seen on the slides. The antigen test was repeated, and the results were negative in duplicate. The report for the MAF stain was corrected, and the physicians were

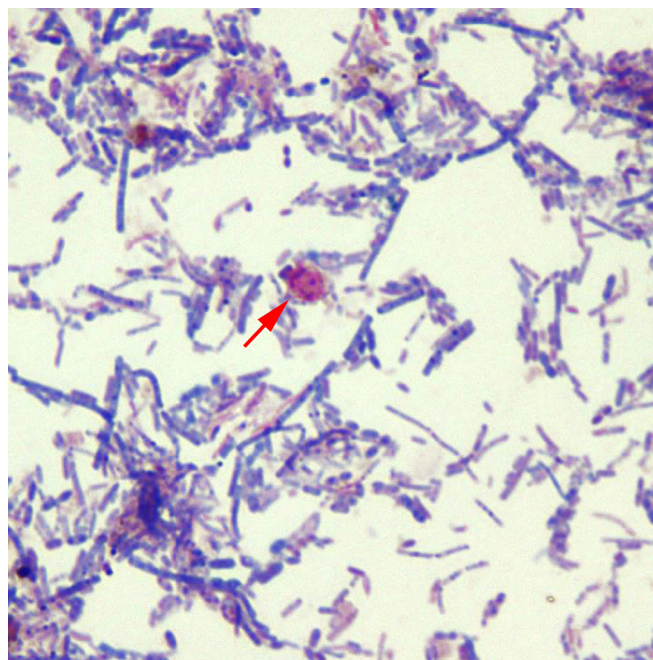


FIG 1 Modified acid-fast stain of formalin-fixed stool, revealing a rare *Cryptosporidium* oocyst (arrow) (magnification,  $\times 1,000$ ).

consulted such that the management of cryptosporidiosis was incorporated into the long-term care of the patient.

#### DISCUSSION

*Cryptosporidium* is an apicomplexan protozoan infecting the gastrointestinal tract of animals and humans, whose phylogenetic placement and life cycle biology are still being interrogated (1). Infection occurs via fecal-oral transmission upon ingestion of oocysts. Each oocyst releases four sporozoites that develop into trophozoites in the gut (1). The trophozoites engage the apical side of the intestinal epithelium and fuse their plasma membrane with the host cell's membrane, forming a parasitophorous vacu-

Citation Rossi A, Couturier MR. 2016. Cryptosporidiosis in a severely immunocompromised HIV patient. J Clin Microbiol 54:2219–2221. doi:10.1128/JCM.00212-16.

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ole. Encapsulated trophozoites then absorb the content of the parasitized cells using a specialized feeder organelle (1). Multiplication of trophozoites via asexual replication is followed by development of micro- and macrogametes. These fuse to give rise to diploid oocysts that are directly infective upon release in the environment via defecation (1).

Cryptosporidiosis is a significant cause of morbidity and mortality in AIDS patients (2). In this group, the prevalence of chronic diarrhea attributable to *Cryptosporidium* dramatically increases in subjects with fewer than 200 CD4<sup>+</sup> T cells/ $\mu$ l (2). In the case presented here, cryptosporidiosis was high on the list of differential diagnoses, as evidenced by the redundant testing that was ordered to rule out *Cryptosporidium*, consisting of microscopic examination of stool with MAF staining, antigen detection, and real-time PCR.

“O&P” is a generic term used to refer to the microscopic examination of all intestinal parasites from stools; however, the conventional implication of the term for North American laboratories is of a concentrated wet mount and a trichrome stain. Detection of *Cryptosporidium* cannot be attained reliably with trichrome stain; instead, a MAF stain of the fecal smears must be performed, and three or more samples collected over a week are recommended for optimal detection (3). This procedure allows the differentiation of the parasite’s oocysts, which appear as fuchsia-stained spheroidal structures with a diameter of 4 to 6  $\mu$ m (Fig. 1). Another common microscopic characteristic of *Cryptosporidium* is the variable presence of partially stained oocysts that appear as “ghosts” (3). While O&P detection of *Cryptosporidium* is still widely used, its clinical sensitivity approaches only 55% (4). Weber et al. showed that the lower limit of detection (LoD) of MAF staining is about 5,000 oocysts per gram of liquid stool, corresponding to a calculated number of 12 parasites per slide (5). This impressive number illustrates how, in situations where the parasite load is low, a successful O&P detection depends on the number of microscopic fields examined and the competency of the technologist. In our case, it took the careful examination of three slides (which was beyond our standard operating procedure), despite excellent technical expertise in our laboratory.

Our initial failure to microscopically identify *Cryptosporidium* infection was complemented by the use of multiplex real-time PCR. PCR tests have been shown to display greater sensitivities than microscopy (2, 6). We used a multiplex real-time PCR assay, developed in our laboratory (unpublished), for the detection of *E. histolytica*, *Giardia*, *C. parvum*/*C. hominis*, *Cyclospora cayentanensis*, and *Dientamoeba fragilis*. This test is based on the amplification of multicopy targets (18S rRNA loci for *Cryptosporidium*). In our in-house validation, we calculated the LoD for the assay (defined as the lowest DNA concentration at which amplification is reproducibly achieved in all replicates) at approximately 17,000 copies of target per ml of stool. Considering that the genome of diploid *C. parvum* oocysts has previously been estimated to contain 20 copies of the 18S rRNA locus (7), we can extrapolate that our PCR assay is able to detect as few as 850 parasites/g of stool with 100% confidence. Given the conservative definition of this LoD value, it is certainly possible that a lower number of oocysts could be detected in a clinical sample.

*Cryptosporidium* antigen was not detected in the patient’s stool with the use of a commercial capture enzyme-linked immunosorbent assay (ELISA). The product we used, according to the manufacturer’s package insert, has a reported 97.7% agreement with

microscopic detection via direct immunofluorescence, which has been shown to be almost twice as sensitive as MAF staining (4). Despite the perceived high sensitivity, *Cryptosporidium* antigen could not be detected in the sample, despite the testing of an aliquot of stool with and without fixative from multiple submissions. However, in the absence of stated LoD values and details on the capture antibody of this kit, we cannot elaborate on the causes of this negative result. Nonetheless, it is worth mentioning that we have encountered antigen-negative samples that test positive by MAF staining or PCR assay on multiple occasions. We previously encountered a patient in which three MAF stains were ordered on separate stools and were all positive for *Cryptosporidium* oocysts even though only one of three samples tested positive for the antigen. In a retrospective study comparing PCR for gastrointestinal (GI) pathogens to conventional testing (unpublished data), three cases of *Cryptosporidium* infection that were also tested for antigen were detected by PCR. Only one of these samples was positive by antigen testing. None of these cases had MAF stains ordered.

It has previously been shown that discrepant results between O&P detection, antigen detection, and PCR are not uncommon (2, 6). While this case and our own research data reiterate this potential diagnostic gap, PCR appears to be the methodology with the greatest sensitivity. This attribute, coupled with the rapid turnaround time (TAT) and the amenability to multiplexing, makes PCR a primary choice in the management of chronic diarrhea in critically ill patients. In this respect, several new platforms capable of detecting *Cryptosporidium* and other protozoa and enteric pathogens are becoming increasingly popular (3) and should be considered first-line testing for high-risk patients, given the excellent sensitivity, specificity, and TAT of these platforms.

## SELF-ASSESSMENT QUESTIONS

1. In what patient population is *Cryptosporidium* expected to be the most common cause of severe, complicated diarrhea?
  - (a) Immunocompetent adults
  - (b) Immunocompetent children
  - (c) Severely immunosuppressed AIDS patients with fewer than 200 CD4<sup>+</sup> cells/ $\mu$ l
  - (d) HIV-positive patients with more than 200 CD4<sup>+</sup> cells/ $\mu$ l
2. What is the recommended staining method for microscopic identification of *Cryptosporidium*?
  - (a) Trichrome staining
  - (b) Modified trichrome staining
  - (c) Modified acid-fast staining
  - (d) Giemsa staining
3. Which method is the least sensitive for detecting *Cryptosporidium*?
  - (a) Microscopic examination with MAF staining
  - (b) PCR
  - (c) Stool antigen detection
  - (d) Direct immunofluorescence

## REFERENCES

1. Clode PL, Koh WH, Thompson RC. 2015. Life without a host cell: what is *Cryptosporidium*? *Trends Parasitol* 31:614–624. <http://dx.doi.org/10.1016/j.pt.2015.08.005>.
2. Kaushik K, Khurana S, Wanchu A, Malla N. 2008. Evaluation of staining techniques, antigen detection and nested PCR for the diagnosis of cryptosporidiosis in HIV seropositive and seronegative patients. *Acta Trop* 107: 1–7. <http://dx.doi.org/10.1016/j.actatropica.2008.02.007>.
3. McHardy IH, Wu M, Shimizu-Cohen R, Couturier MR, Humphries RM. 2014. Detection of intestinal protozoa in the clinical laboratory. *J Clin Microbiol* 52:712–720. <http://dx.doi.org/10.1128/JCM.02877-13>.
4. Alles AJ, Waldron MA, Sierra LS, Mattia AR. 1995. Prospective comparison of direct immunofluorescence and conventional staining methods for detection of *Giardia* and *Cryptosporidium* spp. in human fecal specimens. *J Clin Microbiol* 33:1632–1634.
5. Weber R, Bryan RT, Bishop HS, Wahlquist SP, Sullivan JJ, Juranek DD. 1991. Threshold of detection of *Cryptosporidium* oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods. *J Clin Microbiol* 29:1323–1327.
6. Morgan UM, Pallant L, Dwyer BW, Forbes DA, Rich G, Thompson RC. 1998. Comparison of PCR and microscopy for detection of *Cryptosporidium parvum* in human fecal specimens: clinical trial. *J Clin Microbiol* 36:995–998.
7. Le Blancq SM, Khramtsov NV, Zamani F, Upton SJ, Wu TW. 1997. Ribosomal RNA gene organization in *Cryptosporidium parvum*. *Mol Biochem Parasitol* 90:463–478. [http://dx.doi.org/10.1016/S0166-6851\(97\)00181-3](http://dx.doi.org/10.1016/S0166-6851(97)00181-3).